

MRID No. 422563-04

DATA EVALUATION RECORD

1. **CHEMICAL:** NTN 33893. *129099*
Shaughnessey No. ~~129059~~
2. **TEST MATERIAL:** NTN 33893 technical; 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-*N*-nitro-1*H*-imidazol-2-amine; CAS No. 105827-78-9; Batch No. 9030211; 95.0% active ingredient; a tan powder.
3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: Midge (*Chironomus tentans*).
4. **CITATION:** Gagliano, G.G. 1991. Growth and Survival of the Midge (*Chironomus tentans*) Exposed to NTN 33893 Technical Under Static Renewal Conditions. Report No. 101985. Prepared by Mobay Corporation, Stilwell, KS. Submitted by Mobay Corporation, Kansas City, KS. EPA MRID No. 422563-04.
5. **REVIEWED BY:**

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Signature: *Louis M. Rifici*
Date: *9/28/92*
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6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
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Signature: *P. Kosalwat*
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7. **CONCLUSIONS:** In this test, only the initial 48-hour period is "core". The remainder of the test is invalid because the dilution water control and solvent control appear to have been contaminated with the test material. The 48-hour LC₅₀ value of 68.9 µg/l (mean measured concentration) classifies NTN 33893 as highly toxic to midge larvae. The 48-hour NOEC was 1.04 µg/l mean measured concentration.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**



10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Second instar (12 days post-hatch) midge larvae (*Chironomus tentans*) were obtained from in-house cultures maintained in hard blended water. The cultures were fed a suspension of Tetramin® and cereal leaves five times per week. The temperature and photoperiod during culturing were $22 \pm 1^{\circ}\text{C}$ and 16 hours of light.
- B. Test System: Vessels used in the test were 1-l glass beakers containing 900 ml of test solution. Silica sand was used to provide a substrate depth of 0.5-1 mm. The beakers were randomly positioned in a water bath under a 16-hour light/8-hour dark photoperiod. Light intensity ranged from 40-60 ft-candles. Thirty-minute dawn and dusk simulations were used.

The primary stock solution (20 g a.i./l) was prepared by dissolving 2.1048 g of NTN 33893 in 100 ml of dimethylformamide (DMF) at 22°C . Three additional stocks were prepared by serial dilution. The test solutions were prepared by mixing an appropriate volume of appropriate stock with 1 l of dilution water.

The dilution water used was hard blended water (a mixture of treated city water and spring water) with a hardness of 118 mg/l, an alkalinity of 83 mg/l, and a pH of 8.1-8.2. The chlorine content of the water was monitored continuously to assure the residual chlorine remained $<3 \mu\text{g/l}$.

- C. Dosage: Ten-day static-renewal test. Based on a preliminary test, seven nominal concentrations (0.33, 1.0, 3.0, 10, 33, 100, and $300 \mu\text{g a.i./l}$), a solvent control ($16.5 \mu\text{l DMF/l}$), and a dilution water control were used.
- D. Design: Ten midge larvae were randomly placed in each replicate chamber, two replicates per concentration. The loading was approximately 1 midge/90 ml. Test solutions were renewed every Monday, Wednesday, and Friday by siphoning the old test solutions out of the test chambers to a depth of approximately 1 cm. Fresh solutions were slowly added to avoid disturbing the test organisms. The fresh solutions were no more than 4 hours old at the time of renewal. The midges were

fed the same food used in culturing at a rate of 0.5 ml/l of test solution.

All beakers were observed once every 24 hours for mortality and abnormal effects. At the end of the test, the midges were grouped by replicate, dried at 60°C for 24 hours, and weighed. The temperature, dissolved oxygen concentration (DO), conductivity, and pH were measured in alternating replicates of the control, solvent control, and the low, middle, and high concentrations on days 0, 3, 5, 7, and 10. The temperature of a centrally-located test beaker was also monitored continuously using a data logging device.

Samples of fresh test solutions were taken on days 0 and 5 to measure actual exposure concentrations. Old test solutions were analyzed on days 3 and 10. The concentration of NTN 33893 was determined using liquid chromatography.

- E. **Statistics:** Dilution water control and solvent control growth data were compared using a t-test. All data were tested for normality (chi-square test) and homogeneity of variances (Bartlett's test). Survival data were analyzed using Fisher's Exact test. Test levels with significantly lowered survival were excluded from further analyses. Growth data were analyzed using one-way analysis of variance (ANOVA) and Dunnett's test. The 24, 48, 72, 96, and 240-hour LC₅₀ values and associated 95% confidence intervals were determined using a computer program developed by Stephan et al. (1978).

12. **REPORTED RESULTS:** No undissolved test substance was observed in the test chambers during the test. The mean measured concentrations were 0.67, 1.24, 3.39, 10.2, 34.5, 102, and 329 µg a.i./l (Table 2, attached). These values represented 99-203% of nominal concentrations. The control solutions were contaminated with the test material on three of five occasions. The average concentration in the dilution water control and solvent control was 0.20 and 0.15 µg/l, respectively. "No biological effects were observed in the controls and possible contamination of the samples may have occurred during sample extraction."

The mortality of midge larvae are given in Table 3 (attached). The 96-hour LC₅₀ was 10.5 µg/l mean measured concentration (95% C.I. = 7.69-14.4 µg/l) using the probit method. The slope of the toxicity curve was 3.3. The 96-

hour no-observed-effect concentration (NOEC), based on the lack of abnormal effects, was 1.24 $\mu\text{g/l}$.

After 10 days, survival at 3.39 $\mu\text{g/l}$ was significantly lower than pooled control survival (Table 5, attached). Growth was significantly affected at 1.24 $\mu\text{g/l}$. The NOEC, based on survival and growth after 10 days was therefore 0.67 $\mu\text{g/l}$. The 10-day LC_{50} was 3.17 $\mu\text{g/l}$ (95% C.I. = 1.24-10.2 $\mu\text{g/l}$).

On day 0 through 7, the DO ranged from 5.8 to 7.9 mg/l or 79 to 108% of saturation at 20°C. However, on day 10, DO was 2.0-4.0 mg/l "possibly due to an increased oxygen demand created by increased food in the test chambers" (Table 7, attached). The pH values ranged from 7.1 to 8.8. The temperature was 20.8-22.3°C.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The authors presented no conclusions.

Quality Assurance and Study Compliance Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Test Procedure:** The test design differed from the SEP for freshwater invertebrate acute tests. Significant deviations are as follows:

This test was designed to gather survival and growth data, therefore, the midge larvae were fed during testing. The duration should have been 48 hours eliminating the addition of food to the vessels.

The test concentrations were approximately 30% of the next highest concentration. The SEP recommends that each nominal concentration be at least 60% of next highest.

The test solutions were as old as 4 hours at the time of renewal. The SEP states that the test solutions should be used within 30 minutes of preparation.

The DO at test termination ranged from 2.0 to 4.0 mg/l (22 to 43% of saturation at 20°C). Dissolved oxygen levels must remain above 40% of saturation during the test.

The author stated that conductivity was measured in alternating replicates of the control, solvent control, low, middle, and high concentration on days 0, 3, 5, 7, and 10. The results were not presented in the report.

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal program and mean measured concentrations to determine the 48, 96, and 240-hour LC_{50} values (see attached printouts 1-3). The results were similar to those of the author's.

Growth and survival at test termination were analyzed to verify the author's 10-day NOEC. Survival at concentrations $\geq 3.39 \mu\text{g/l}$ was significantly lower than survival in the solvent control (see attached printout 4). Average dry weight of surviving midges at concentrations $\geq 1.24 \mu\text{g/l}$ was significantly lower than the solvent control (see attached printout 4). These results are the same as those of the author's.

- C. **Discussion/Results:** In this test, only the initial 48-hour period is "core". The remainder of the test is invalid because the dilution water control and solvent control appear to have been contaminated with the test material. The 48-hour LC_{50} value of $68.9 \mu\text{g/l}$ (mean measured concentration) classifies NTN 33893 as highly toxic to midge larvae. The 48-hour NOEC was $1.04 \mu\text{g/l}$ mean measured concentration.

- D. **Adequacy of the Study:**

- (1) **Classification:** Core for the initial 48-hour period only.
- (2) **Rationale:** The remainder of the test is invalid because the dilution water control and solvent control appear to have been contaminated with the test material.
- (3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 09-16-92.

TABLE 2

Measured Test Concentrations of NTN 33893 Technical

Nominal Concentration ($\mu\text{g/L}$; ppb)	Day 0	3-day mean measured concentration Measured Concentration ($\mu\text{g/L}$)					Percent of Nominal
		Day 3*	Day 5	Day 7*	Day 10*	Mean ($\pm\text{SD}$)	
Control	ND <0.1	ND <0.1	0.16	0.28	0.15	0.20 (0.07)	-----
Sol. Control	ND <0.1	0.11	0.17	ND <0.1	0.18	0.15 (0.04)	-----
0.33	0.48	0.59 .54	0.94	N/A	0.68	0.67 (0.20)	203
1.0	1.06	1.02 1.04	1.46	N/A	1.43	1.24 (0.23)	124
3.0	3.21	2.96 3.09	3.16	N/A	4.23	3.39 (0.57)	113
10	10.4	10.2 10.3	10.1	N/A	10.2	10.2 (0.12)	102
33	35.4	33.2 34.3	35.0	-----	-----	34.5 (1.2)	99
100	107	97.5 102.3	-----	-----	-----	102 (6.7)	102
300	335	324 329.5	-----	-----	-----	329 (7.8)	110

* = These samples were the old test solutions prior to renewal.

N/A = Not Analyzed

SD = Standard Deviation

TABLE 3

CUMULATIVE MORTALITY OF CHIRONOMIDS
EXPOSED TO NTN 33893 TECHNICAL

Measured Concentration ($\mu\text{g/L}$; ppb)	Cumulative Number of Dead Organisms										
	Study Day										
	0	1	2	3	4	5	6	7	8	9	10
Control	0	0	0	0	0	0	0	1	1	1	1
Sol Control	0	0	0	0	0	0	0	0	0	0	0
0.67	0	0	0	0	0	0	0	0	0	0	0
1.24	0	0	0	0	0	0	0	0	0	0	0
3.39*	0	0	1	1	1	2	2	3	4	10	11
10.2*	0	1	1	1	10	13	17	17	18	19	20
34.5*	0	1	7	16	19	20	--	--	--	--	--
102*	0	0	8	15	20	--	--	--	--	--	--
329*	0	3	20	--	--	--	--	--	--	--	--

* - Significantly different, ($P < 0.05$) compared to other test levels and controls.

TABLE 5

DRY WEIGHT OF SURVIVING CHIRONOMIDS
EXPOSED TO NTN 33893 TECHNICAL

Measured Concentration ($\mu\text{g/L}$; ppb)	REP	Number of Chironomids Surviving	Total Dry Weight of Surviving Chironomids	Average Dry Weight of Each Chironomid
Control	I	10	0.0196 g	1.96 mg
	II	9	0.0193 g	2.14 mg
Sol. Control	I	10	0.0226 g	2.26 mg
	II	10	0.0216 g	2.16 mg
Pooled Controls		39	0.0831	2.13 mg
0.67	I	10	0.0208 g	2.08 mg
	II	10	0.0204 g	2.04 mg
1.24*	I	10	0.0152 g	1.52 mg
	II	10	0.0192 g	1.92 mg
3.39*	I	5	0.0021 g	0.42 mg
	II	4	0.0015 g	0.38 mg

* - Significantly different ($P < 0.05$) from pooled controls.

TABLE 7

Dissolved Oxygen and pH Measurements During the
10-Day Exposure of *Chironomus tentans* to NTN 33893 Technical

Nominal Concentrations ($\mu\text{g/L}$; ppb)	Day 0		Day 3		Day 5		Day 7		Day 10	
	DO	pH	DO	pH	DO	pH	DO	pH	DO	pH
Control	7.6	8.1	6.8	7.8	6.3	7.1	6.6	7.7	2.0	7.3
Sol. Control	7.4	8.8	7.1	7.9	6.7	7.4	5.8	7.7	3.4	7.4
0.33	7.0	8.8	7.3	7.8	7.1	7.5	7.0	7.8	3.0	7.4
10*	7.3	8.8	7.3	7.8	7.3	7.6	7.2	7.9	4.0	7.5
300**	7.4	8.8	7.2	7.9	---	---	---	---	---	---

DO = Dissolved oxygen in mg/L.

* - Water quality parameters were measured in the 3 $\mu\text{g/L}$ test level on Day 10 to due the complete mortality of test organisms in the 10 $\mu\text{g/L}$ test level.

** - Water quality parameters were measured in the 100 $\mu\text{g/L}$ test level on Day 3 to due the complete mortality of test organisms in the 300 $\mu\text{g/L}$ test level.

day 3 mean measured concentrations *48-hour LC50*

RIFICI NTN 33893 CHIRONOMUS TENTANS 09-16-92

CONC	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
329.5	20	20	100	9.536742E-05
102.3	20	8	40	25.17223
34.3	20	7	35	13.1588
10.3	20	1	5	2.002716E-03
3.09	20	1	5	2.002716E-03
1.04	20	0	0	9.536742E-05
.54	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 10.3 AND 329.5 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 118.3196

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	6.572961E-02	← 68.94127	49.35569 - 98.45378

98.45378

LC50 9/16/91

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1061265	1	7.839239E-02

SLOPE = 1.690331

95 PERCENT CONFIDENCE LIMITS = 1.139671 AND 2.240991

LC50 = 68.87281

95 PERCENT CONFIDENCE LIMITS = 44.81775 AND 111.5195

LC10 = 12.21

95 PERCENT CONFIDENCE LIMITS = 5.017858 AND 20.75928

96-hour LC₅₀

RIFICI NTN 33893 CHIRONOMUS TENTANS 09-16-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
329	20	20	100	9.536742E-05
102	20	20	100	9.536742E-05
34.5	20	19	95	2.002716E-03
10.2	20	10	50	58.80985
3.39	20	1	5	2.002716E-03
1.24	20	0	0	9.536742E-05
.67	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 3.39 AND 34.5 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC₅₀ FOR THIS SET OF DATA IS 10.2

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC ₅₀	95 PERCENT CONFIDENCE LIMITS
6	5.135013E-02	← 11.43877	7.679475 - 17.03832

~~17.03832~~

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1373512	1	.9999185

SLOPE = 3.310458
95 PERCENT CONFIDENCE LIMITS = 2.083571 AND 4.537344

LC₅₀ = 10.45897
95 PERCENT CONFIDENCE LIMITS = 7.686511 AND 14.42776

LC₁₀ = 4.323704
95 PERCENT CONFIDENCE LIMITS = 2.343523 AND 6.104206

10-day LC50

RIFICI NTN 33893 CHIRONOMUS TENTANS 09-16-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
329	20	20	100	9.536742E-05
102	20	20	100	9.536742E-05
34.5	20	20	100	9.536742E-05
10.2	20	20	100	9.536742E-05
3.39	20	11	55	41.19014
1.24	20	0	0	9.536742E-05
.67	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.24 AND 10.2 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.172205

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

MIDGE SURVIVAL AFTER 10 DAYS

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	0.67 µg/l	20	0	
2	1.24	20	0	
3	3.39	20	11	*
4	10.2	20	20	*
5	34.5	20	20	*
6	102	20	20	*
7	329	20	20	*

422563-04, NTN 33893, midge dry weight

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	2.2100	CALCULATED t VALUE =	1.5541
GRP2 (BLANK CRTL) MEAN =	2.0500	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	0.1600		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05

TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

Shapiro Wilks test for normality

Data PASS normality test at P=0.01 level. Continue analysis.

Bartlett's test for homogeneity of variance

Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	4.404	1.101	53.548
Within (Error)	5	0.103	0.021	
Total	9	4.507		

Critical F value = 5.19 (0.05,4,5)

Since F > Critical F REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	2.210	2.210		
2	dilution contrl	2.050	2.050	1.116	
3	0.67 µg/l	2.060	2.060	1.046	
4	1.24	1.720	1.720	3.417	*
5	3.39	0.400	0.400	12.623	*

Dunnett table value = 2.85 (1 Tailed Value, P=0.05, df=5,4)

DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	2			
2	dilution contrl	2	0.409	18.5	0.160
3	0.67 µg/l	2	0.409	18.5	0.150
4	1.24	2	0.409	18.5	0.490
5	3.39	2	0.409	18.5	1.810

TITLE: 422563-04, NTN 33893, midge dry weight
FILE: a:42256304.dt1
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 5

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	2.2600	2.2600
1	solvent control	2	2.1600	2.1600
2	dilution contrl	1	1.9600	1.9600
2	dilution contrl	2	2.1400	2.1400
3	0.67 µg/l	1	2.0800	2.0800
3	0.67 µg/l	2	2.0400	2.0400
4	1.24	1	1.5200	1.5200
4	1.24	2	1.9200	1.9200
5	3.39	1	0.4200	0.4200
5	3.39	2	0.3800	0.3800
